REPORT DOCUMENTATION P

AD-A240 119

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	7 Aug 91	ANNUAL 15 May 90 to 14 May 91

4. TITLE AND SUBTITLE

CYTOCHEMICAL ORGANIZATION OF THE RETINO-SUPRACHIASMATIC SYSTEM

5. FUNDING NUMBERS

GR - AFOSR-90-0072
PE - 61102F
PR - 2312
TA - A3

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AFOSR-TR 91 0704

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10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release;
distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

We have completed a number of studies of the suprachiasmatic nucleus (SCN) and associated hypothalamic regions. This part of the brain is involved in regulation of circadian rhythms. Work included ultrastructural immunocytochemistry, intracellular electrophysiology, fluo-3 calcium imaging of SCN cells, and experimental neuroanatomy.

91 9 [illegible]

14. SUBJECT TERMS	15. NUMBER OF PAGES
	3
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
(U)	(U)	(U)	UNLIMITED

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Progress Report: AFOSR P.I. Anthony N. van den Pol, PhD
Chronobiology Program/ Year 1 ending May 1991 July 25,1991

Dear Dr.Haddad:

Enclosed please find our progress report for the first year of support from the AFOSR in the Chronobiology Program which ended in May,1991. Thankyou for taking the time to visit our lab at Yale during your recent trip to the Northeast.

We have completed a number of studies of the suprachiasmatic nucleus (SCN) and associated hypothalamic regions. This part of the brain is involved in regulation of circadian rhythms. Work included ultrastructural immunocytochemistry, intracellular electrophysiology, fluo-3 calcium imaging of SCN cells, and experimental neuroanatomy.

Using antisera against GABA and its synthesizing enzyme glutamate decarboxylase, we have studied the ultrastructural morphology and density of GABAergic neurons in the suprachiasmatic nucleus (SCN) and related medial hypothalamic areas. We found that presynaptic boutons which were immunoreactive for GABA or for glutamate decarboxylase made up almost half of all boutons. This result indicates that GABA may play a very important role in the modulation of neuronal activity with the SCN, and that since GABA is an inhibitory transmitter, inhibitory synapses account for at least half of all synaptic events.

We also have raised an antibody against the excitatory amino acid neurotransmitter glutamate. The antibody appears to be specific for glutamate by immunodot blot, ELISA, Western blot, and epoxy conjugated Sepharose beads. When used in the hypothalamus, we found many presynaptic axons in the SCN and other regions of the hypothalamus that showed positive immunoreactivity for glutamate. The ratio of immunogold particles over highly immunoreactive axons compared to postsynaptic dendrites was similar to that found in other regions of the brain where physiological studies have suggested glutamatergic neurotransmission exists.

In collaboration with Drs. J.P.Wuarin and Ed Dudek who are also supported by the AFOSR, we made intracellular electrical recordings from neuroendocrine neurons of the arcuate nucleus and paraventricular nucleus (which receives axonal projections from the SCN). and found we could block the majority of the excitatory response to electrically induced axonal neurotransmitter release with the agents CNQX and kynurenic acid. These are both specific glutamate antagonists. When we grew medial hypothalamic cells

in culture, we found we could induce a Ca^{2+} increase in almost all neurons with glutamate and its agonists, kainate and quisqualate. Together these data strongly suggest that the primary excitatory neurotransmitter in the medial hypothalamus and neuroendocrine system is glutamate.

Previous efforts to visualize dendritic arbors indicated that classical Golgi impregnations worked, but many sections needed to be used in order to find a few well-stained cells. We have developed a novel staining method which allows us to routinely stain SCN cells by exerting a slight pressure on the ventral surface of the brain near the optic chiasm, and then with an esterification method, staining the pressure-traumatized SCN cells. An added advantage of this stain is that it allows detection of neurons and their dendritic tree to be visualized even if fixed only a few minutes after injury. This may be of use in models of brain injury where the relative influence of primary and secondary injury are being studied.

Finally, we have invested considerable time in digital video analysis of the response of SCN cells to glutamate and related agonists in cultures of SCN neurons and glia. We find both neurons and glial cells show a strong increase in intracellular Ca^{2+} in the presence of glutamate. Interestingly, ultradian (with a period less than 24 hr) oscillations of Ca^{2+} can be induced by glutamate, particularly in glial cells. Several theoretical models of circadian rhythms have suggested that a circadian oscillator can be made of interacting ultradian oscillators.

Papers publish d during the first year of support from the AFOSR:

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